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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Preservative System for Vitamin E Aqueous Solution

(72) Skeffington, Donald - Canada ;
Siegrist, Hansruedi - Canada ;
Boudjikanian, Adom - Canada ;

(73) Ciba-Geigy Canada Ltd. - Canada ;

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Notice: This application is as filed and may therefore contain an incomplete specification.

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PRESERVATIVE SYSTEM FOR VITAMIN E AQUEOUS SOLUTION

ABSTRACT

The invention relates to pharmaceutical formulations for water soluble Vitamin E. The formulations may include methyl parabens, propyl parabens, potassium sorbate, propylene glycol, sodium citrate, citric acid, EDTA and tocopheryl polyethylene glycol succinate (TPGS).

The invention relates to a formulation for a pharmaceutical product. In particular, the invention relates to a preservative system for water soluble Vitamin E.

Vitamin E, has traditionally been known as a fat soluble vitamin which exists in a variety of forms, one of the most active of which is d-alpha-tocopherol. Although the exact biological function of Vitamin E in humans is unknown, it is considered an essential element of human nutrition. Many of its therapeutic actions are related to its antioxidant properties. Vitamin E is naturally present in many foods, particularly in cereals, nuts, and leafy green and yellow vegetables. As it is fat soluble in its traditional form, it is stored in the fat fractions of animal tissues and hence significant sources of Vitamin E also include eggs and meat. Absorption of Vitamin E from the gastrointestinal tract depends on the presence of bile. As a result, individuals who, as a result of genetic deficiency, pathological condition or otherwise, cannot absorb fat, are unable to utilize the traditional fat soluble Vitamin E present in many foods. For example, individuals afflicted with the disease cystic fibrosis, are unable to effectively absorb fat from their gastrointestinal tract. Although Vitamin E is available in a formulation suitable for parenteral administration (i.e. IM Injection) such products are expensive and not always convenient or easy to administer.

In the 1950's Eastman Kodak Company developed a water soluble form of Vitamin E now known as Tocopheryl Polyethylene Glycol 1000 Succinate, abbreviated TPGS. Reference is made to U.S. Patent No. 2,680,749. Although the aforesaid U.S. patent mentions (column 3, lines 53-55) that water solutions of the tocopheryl derivatives can be used for oral administration, further information is not provided. It was recognized early on, by Eastman and others, that the combination of TPGS and water would provide a

medium in which molds and other micro-organisms would readily grow and flourish. Although Eastman considered the problem of developing a formulation for oral administration which included TPGS and which had a suitable shelf life, Eastman was unsuccessful in overcoming the problem. To date, some forty years after the Eastman invention, notwithstanding the significant demand for water soluble Vitamin E (TPGS) amongst cystic fibrosis patients and others who for whatever reason cannot absorb fat, no one has been able to formulate, to the knowledge of the inventors, a product for oral administration, incorporating TPGS, which has an acceptable shelf life.

The inventors have, after a lengthy period of study and experimentation, developed a pharmaceutical formulation and a preservative system which incorporates TPGS and has a shelf life suitable for its intended use, and which meets the regulatory requirements of the Health Protection Branch, Government of Canada.

The present invention provides a pharmaceutical formulation comprising:

water soluble Vitamin E (TPGS) 20% w/v

potassium sorbate 0.200% w/v

methyl paraben 0.128% w/v

propyl paraben 0.032% w/v

sodium citrate 0.877% w/v

citric acid 0.429% w/v

distilled water to 100 ml

wherein the pH is 5.0.

In a further embodiment the invention provides for a pharmaceutical formulation comprising:

TPGS 20% w/v

potassium sorbate 0.20% w/v

methyl paraben 0.128% w/v

propyl paraben 0.032% w/v

propylene glycol 10.0% w/v

sodium citrate 0.877% w/v

citric acid 0.429% w/v

distilled water to 100 ml

wherein the pH is 5.0.

In yet a further embodiment the present invention provides a pharmaceutical formulation comprising:

TPGS 20% w/v

potassium sorbate 0.2% w/v

propylene glycol 10% w/v

sodium citrate 0.877% w/v

citric acid 0.429% w/v

distilled water to 100 ml

wherein the pH is 5.0.

The problem facing the inventors was to develop a preservative system and a pharmaceutical formulation for water soluble Vitamin E (TPGS), namely a solution, which was shelf stable, which met pharmaceutical formularly standards, Canadian government

standards, and resulted in a product that was safe and effective for human consumption. The preservative system and pharmaceutical formulation had to be designed to be suitable and acceptable for oral ingestion.

EXPERIMENTATION AND RESULTS

Amongst experiments conducted were the following.

A series of Vitamin E solutions containing 75 units per millilitre were prepared with different preservative systems and evaluated for their antimicrobial preservative effectiveness, using USP XXI procedures. TPGS available from Eastman was used as the Vitamin E source. No assay was performed to determine the content of the Vitamin E international units. During preparation of the various solutions the TPGS was melted first at 50°C and maintained at this temperature. A 30 gram amount of TPGS was used in all cases. In a separate beaker the preservatives were dissolved in 120 millilitres of water maintained at 85°C. The TPGS was always poured into the water phase under constant stirring. Then the volume was made up to 150.00 ml using water, at room temperature.

Six sample formulations were prepared (A,B,C,D,E,F). pH adjustments were made on samples B, D and F, using citric acid and sodium citrate. Being an esterfied molecule, TPGS is likely to undergo hydrolysis to some extent. In order to minimize this reaction and improve the overall stability of formulae the buffer (citric acid/sodium citrate) must be dissolved in the initial quantity of water, before the other ingredients. All preservative systems and levels used were acceptable to the Health Protection Branch.

The following sample formulations were prepared:

SAMPLE A

Distilled Water	q.s. to	150.00 ml
Methyl Paraben		0.27 Grams
Propyl Paraben		0.03 Grams
TPGS		30.00 Grams

pH = 4.8

SAMPLE B

Same as Sample A, but pH = 3.5

SAMPLE C

Distilled Water	q.s. to	150.00 ml
Methyl Paraben		0.12 Grams
Potassium Sorbate		0.06 Grams
TPGS		30.00 Grams

pH = 5.5

SAMPLE D

Same as Sample C, but pH = 3.9

SAMPLE E

Distilled Water	q.s. to	150.00 ml
Methyl Paraben		0.12 Grams
Potassium Benzonate		0.06 Grams
TPGS		30.00 Grams

pH = 5.0

SAMPLE F

Same as Sample E, but pH = 3.9

Test Organisms

The following organisms were used in evaluating the different sample formulations:

1)	Candida albicans	ATCC 10231
2)	Aspergillus niger	ATCC 16404
3)	Escherichia coli	ATCC 8739
4)	Pseudomonas aeruginosa	ATCC 9027
5)	Staphylococcus aureus	ATCC 6538

Procedure

A 20 millilitre sample of each formulation was transferred to each of the five sterile capped bacteriological tubes of suitable size. Each tube was inoculated with one of the standardized microbial suspensions of the above organisms using a ratio equivalent to 0.10 millilitre of inoculum to 20 millilitres of product. The concentrations of the organisms in the test preparation were standardized after inoculation to 500,000 per millilitre. The inoculated tubes were incubated at 20° to 25°C. Each of the containers was examined at 7, 14, 21 and 28 days, subsequent to inoculation. When a formula appeared to fail the requirements of the test, further testing was suspended.

The results for all the sample formulas were as follows:

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	390,000	480,000	--*	--
A. niger	500,000	180,000	--	--	--
E. coli	500,000	More than 3,000,000	7,600,000	--	--
P. aeruginosa	500,000	2,275,000	14,300,000	--	--
S. aureus	500,000	2,550,000	More than 300,000	--	--

*Further testing was suspended.

SAMPLE B

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	590,000	625,000	480,000	499,000
A. niger	500,000	100,000	115,000	110,000	60,000
E. coli	500,000	88,500	9,100	135	NIL
P. aeruginosa	500,000	NIL	NIL	NIL	NIL
S. aureus	500,000	500	NIL	NIL	NIL

SAMPLE C

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	67,500	79,000	—*	—
A. niger	500,000	170,000	--	--	--
E. coli	500,000	1,310,000	111,000	--	—
P. aeruginosa	500,000	660,000	More than 3,000,000	--	—
S. aureus	500,000	1,650,000	More than 300,000	--	--

*Further testing suspended.

SAMPLE D

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	68,500	90,000	168,000	91,000
A. niger	500,000	240,000	85,000	250,000	255,000
E. coli	500,000	1,050	NIL	NIL	NIL
P. aeruginosa	500,000	NIL	NIL	NIL	NIL
S. aureus	500,000	1,400	NIL	NIL	NIL

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	260,000	585,000	--*	--
A. niger	500,000	85,000	--	--	--
E. coli	500,000	775,000	300,000	--	--
P. aeruginosa	500,000	340,000	1,200,000	--	--
S. aureus	500,000	45,000	30,000	--	--

*Further testing suspended.

SAMPLE F

NUMBER OF MICROORGANISMS SURVIVING DURING TESTING PERIOD

Organism	Initial Concentration	7th Day	14th Day	21st Day	28th Day
	Microorganisms per Milliliter				
C. albicans	500,000	33,300	59,000	--*	--
A. niger	500,000	155,000	--	--	--
E. coli	500,000	107,300	4,200	--	--
P. aeruginosa	500,000	500,000	1,730,000	--	--
S. aureus	500,000	550	NIL	--	--

Sample formulations A, C, E and F were not acceptable because the concentrations of the identified organisms failed to keep below the acceptable levels suggested in the USP XXI.

In sample formula B, the concentrations of viable bacteria (E coli), although not reduced to more than 99.9% of the initial concentration by the fourteenth day, resulted in no count being detected by the twenty-eighth day. In the same formula Candida albicans failed to remain at or below the initial level during the first fourteen days, but it stayed generally at the initial level by the twenty-eighth day. The results suggested there was a delayed preservative effectiveness as a result of formula B.

Sample formula D also met the requirements of the USP XXI, NT Microbial Preservative Effectiveness test.

Subsequent experimentation with modified formulations using different preservative systems, preservative potentiators and buffers resulted in the identification of the following three preferred embodiments.

Ingredients (% w/v)	Formula 1	Formula 2	Formula 3
T.P.G.S.	20.000	20.000	20.000
Potassium Sorbate, N.F.	0.200	0.200	0.200
Methyl Paraben, N.F.	0.128	0.128	-----
Propyl Paraben, N.F.	0.032	0.032	-----
Propylene Glycol, U.S.P.	-----	10.000	10.000
Sodium Citrate, U.S.P.	0.877	0.877	0.877
Citric Acid, U.S.P.	0.429	0.429	0.429
Purified Water, U.S.P	100.000	100.000	100.000

The pH of formulas 1, 2 and 3 was adjusted to 5.0.

The aforesaid formulas 1, 2 and 3 were subjected to a preservative challenge test according to Ciba-Geigy's method IA-140/1.

The preservative challenge test results for each of formulations 1, 2 and 3 is set out below.

Product	Formula 1				
Microorganism / # days	*E.coli* CFC/ml	*S.aureus* CFC/ml	*P.aeruginosa* CFC/ml	*C.albicans* CFC/ml	*As.niger* CFC/ml
0	14,000,000	26,000,000	1,000,000	70,000	100,000
7	600,000	2,000	<1	300,000	<1
14	9,000	<1	<1	20,000	10
21	<1	<1	<1	10,000	100
28	<1	<1	<1	400	<1
42	<1	<1	<1	<1	<1

The initial suspensions contained approx. 10^6 CFU/ml

Product	Formula 2				
Microorganism / # days	*E.coli* CFC/ml	*S. aureus* CFC/ml	*P.aeruginosa* CFC/ml	*C. albicans* CFC/ml	*As.niger* CFC/ml
0	6,000,000	2,000,000	400,000	700,00	10,000
7	20,000	8,000	<1	240,000	<1
14	100	<1	<1	20,000	10
21	<1	<1	<1	10,000	10
28	<1	<1	<1	3,200	<1
42	<1	<1	<1	<1	<1

The initial suspensions contained approx. 10^6 CFU/ml

Product	Formula 3				
Microorganism / # days	*E.coli* UFC/ml	*S.aureus* UFC/ml	*P.aeruginosa* UFC/ml	*C.albicans* UFC/ml	*As.niger* UFC/ml
0	4,000,000	3,000,000	1,000,000	10,000	10,000
7	400,000	20,000	<1	340,000	<1
14	100	<1	<1	20,000	10
21	<1	<1	<1	15,000	100
28	<1	<1	<1	8,000	100
42	<1	<1	<1	<1	<1

The initial suspensions contained approx. 10^6 CFU/ml

In the table below, a phosphate buffer/peptone solution was used as a diluent in a challenge test. The resulting figures were regarded as "positive control" results.

Product	Phosphate buffer + peptone				
Microorganism / # days	*E.coli* UFC/ml	*S.aureus* UFC/ml	*P.aeruginosa* UFC/ml	*C.albicans* UFC/ml	*As.niger* UFC/ml
0	10,000,000	2,000,000	2,000,000	700,000	20,000
7	28,000,000	30,000,000	30,000,000	12 000 00	10,000
14	30,000,000	300,000,000	130,000,000	2,000,000	30,000
21	300,000,000	350,000,000	>300,000,000	200,000,000	300,000
28	>300,000,000	>300,000,000	>300,000,000	200,000,000	1,000,000
42	—	—	—	—	—

The initial suspensions contained approx. 10^6 CFU/ml

Experiments were also conducted with respect to Edetate Disodium (EDTA). EDTA was found to be useful in the formula of the invention when used in combination with parabens and/or sorbates. In particular, a formula containing 10% w/v propylene glycol and buffered to pH 4 and 5, with EDTA 0.05% w/v combined with parabens or potassium sorbate respectively, eradicated the initial bacterial population virtually totally by the second day and no growth was observed on day seven. In a further trial, buffered to pH 4, where propylene glycol was absent and EDTA was combined with potassium sorbate alone, gave similar results.

With respect to the ingredients used in the formulations of the invention, the inventors make the following comments.

Potassium sorbate is a preservative having anti-bacterial and anti-fungal properties. Potassium sorbate is active in the acidic zone, up to pH 6.5.

Propyl paraben and methyl paraben are esters each of which exhibit preservative properties, comparable to those exhibited by potassium sorbate. The parabens are active within a pH range of 4-8. Their loss in potency in certain cases, mandates the addition of other molecules having preservative activity. In the present case, the spectrum of activity of the paraben esters is increased by the addition of the potassium sorbate. The potassium sorbate is particularly active against molds and yeasts and will compensate any potential deactivation of the parabens.

EDTA is an antioxidant and antibacterial synergist, having also intrinsic antimicrobial activity. When used in combination with parabens and/or sorbates, EDTA will provide a broad spectrum preservative effect including toxic activity against Gram Negative bacteria like Pseudomas sp. EDTA may be used in the formula of the invention,

in the range of 0.025–0.100%.

The citric acid and sodium citrate are used in combination as a buffer. Citric acid is also a sequestering/anti-oxidant agent and hence can further improve the overall stability of the Vitamin E solutions. The concentration of citric acid and sodium citrate used can be varied to some extent, as long as the resulting pH of the formulation is within the range 4–6. The propylene glycol is useful in the formulation of the invention as a solvent and preservative. It is a water miscible co-solvent and an inhibitor of fermentation, and has its own toxic activity against bacteria and fungi. Furthermore, propylene glycol can potentiate the anti-microbial effects of other preservatives. Propylene glycol also acts as a vitamin stabilizer.

The water acts as a solubilizing and diluting vehicle.

In formulas 1 and 2, we have a complex preservative system, where the spectrum of activity of paraben esters is increased by the addition of the potassium sorbate. In formula 2, potentiation of action results due to the other anti-microbials, whereas in formula 3, good results are obtained even when the parabens are excluded. An experimental formulation, containing only 0.2% potassium sorbate, without parabens and propylene glycol, failed to pass the preservative challenge test. The preferred pH is pH 5, using the citric acid/sodium citrate buffer system, as this pH appears to optimize the activity of both parabens and the sorbate.

From the microbiological assays, it may be noted that at 28 days formula 1 was better than formula 2, which in turn was better than formula 3. At 42 days, formulas 1, 2 and 3 were equally good. There appears to be a delayed preservative action from propylene glycol in respect of formulas 2 and 3.

The following ingredients may be used in the invention in the following concentration ranges:

potassium sorbate	0.08–0.20% w/v
methyl paraben	0.00–0.20% w/v (0.00–0.14% preferred)
propyl paraben	0.00–0.20% w/v (0.00–0.05% preferred)
EDTA	0.025–0.100% w/v
propylene glycol	0–25.0% w/v (depending on the amount of the parabens present, 0.00–10.00% is the preferred range)
sodium citrate	0.65–1.25% w/v (0.85–0.93% preferred)
citric acid	0.06–1.50% w/v (0.10–1.20% preferred)

(sodium citrate/citric acid in combination sufficient to stabilize and buffer the pH within the range 4–6)

TPGS up to 20.0% w/v.

In view of the disclosure herein, those skilled in the art will recognize that further and other embodiments of the present invention may be utilized without departing from the spirit and substance of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

1. A shelf stable pharmaceutical formulation for TPGS comprising:

potassium sorbate	0.08–0.200% w/v
propylene glycol	0.0–25.000% w/v
TPGS	up to 20.00% w/v
water	

wherein the pH is in the range of 4–6, utilizing sodium citrate and citric acid as a buffer system.

2. The formulation of claim 1, including methyl paraben in the range of 0.00–0.20% w/v and propyl paraben in the range of 0.00–0.20% w/v.

3. The formulation of claim 2 wherein sodium citrate is in the range of 0.65–1.25% w/v and citric acid is in the range of 0.06–1.50% w/v.

4. The formulation of claim 1, 2 or 3 including EDTA in the range of 0.025–0.100% w/v.

5. The formulation of claim 1, 2 or 3 wherein methyl paraben is present in the range of 0.00–0.14% w/v.

6. The formulation of claim 1, 2 or 3 wherein propyl paraben is present in the range of 0.00–0.05% w/v.

7. The formulation of claim 1, 2 or 3 wherein propylene glycol is present in the range of 0.00–25.00% w/v.

8. The formulation of claim 1, 2 or 3 wherein sodium citrate is present in the range of 0.85–0.93% w/v.

9. The formulation of claim 1, 2 or 3 wherein citric acid is present in the range of 0.10–1.20% w/v.

10. The formulation of claim 1 wherein propylene glycol is present in the amount of 10.00% w/v.

11. The formulation of claim 1 including methyl paraben up to 0.128% w/v and propyl paraben up to 0.032% w/v.

12. The formulation of claim 1, 2 or 3 wherein the pH is 5.

13. A shelf stable pharmaceutical formulation for TPGS comprising:

methyl paraben	0.00–0.20% w/v
propyl paraben	0.00–0.20% w/v
potassium sorbate	0.08–0.20% w/v
propylene glycol	0.00–25.00% w/v
sodium citrate	0.85–1.25% w/v
citric acid	0.10–1.50% w/v
TPGS	up to 20%
water	

14. A shelf stable pharmaceutical formulation for TPGS comprising:

methyl paraben	0.00–0.14% w/v
propyl paraben	0.00–0.05% w/v
potassium sorbate	0.08–0.20% w/v
propylene glycol	0.00–10.00% w/v
sodium citrate	0.65–0.93% w/v
citric acid	0.06–1.20% w/v
TPGS	up to 20%
water	

15. The formulation of claim 13 or 14 including EDTA in the range of 0.025%–0.100% w/v.

16. A shelf stable pharmaceutical formulation for TPGS comprising:

potassium sorbate	0.200% w/v
methyl paraben	0.128% w/v
propyl paraben	0.032% w/v
sodium citrate	0.877% w/v
citric acid	0.429% w/v
TPGS	up to 20.00% w/v
water	

wherein the pH is in the range of 4–6.

17. A shelf stable pharmaceutical formulation for TPGS comprising:

potassium sorbate	0.200% w/v
methyl paraben	0.128% w/v
propyl paraben	0.032% w/v
propylene glycol	10.000% w/v
sodium citrate	0.877% w/v
citric acid	0.429% w/v
TPGS	up to 20.00% w/v
water	

wherein the pH is in the range of 4–6.

18. A shelf stable pharmaceutical formulation for TPGS comprising:

potassium sorbate	0.200% w/v
propylene glycol	10.00% w/v

sodium citrate	0.877% w/v
citric acid	0.429% w/v
TPGS	up to 20.00% w/v
water	

wherein the pH is in the range of 4–6.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente